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SUB B1

CLAIMS

1. An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

2. The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

3. The enzyme mixture of claim 2, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

4. The enzyme mixture of claim 1, wherein said second enzyme is a mutant DNA polymerase.

5. The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

6. An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

SUB B2

7. An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme is a Taq DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

8. The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

9. The enzyme mixture of claim 6, 7, or 8, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

10. The enzyme mixture of claim 9, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

SUB B3

11. A kit for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, said second enzyme comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity, and packaging material therefore.

12. The kit of claim 11, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

13. The kit of claim 12, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

14. The kit of claim 13, wherein said second enzyme is a mutant DNA polymerase.

15. The kit of claim 14, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

16. The kit of claim 14, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

17. A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

18. A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a Taq DNA polymerase, and packaging material therefore, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

19. The kit of claim 11, 17, or 18, further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or additive, a control DNA template and a control primer.

20. The kit of claim 14, 17, or 18, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

21. The kit of claim 20, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

SUB B4

22. A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

23. The method of claim 14, wherein said nucleic acid template is a DNA or an RNA molecule.

24. The method of claim 14, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

25. The method of claim 16, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UlTma DNA polymerase, Tli DNA

polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

26. The method of claim 14, wherein said second enzyme is a mutant DNA polymerase.

27. The method of claim 18, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UlTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

28. The method of claim 18, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

29. A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

30. A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

31. The method of claim 27, 29, or 30, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

5 [illegible] synthesized DNA product into a TA cloning vector.

32. The method of claim 28, 30, or 31, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

SUB B5

33. The method of claim 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

34. The method of claim 23, 30 or 31, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

ADD B6